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SACCHARIN

TOXICITY and TERATOGENICITY STUDIES in AVIAN EMBRYOS

FDA CONTRACT #71-330

B.L. Reid

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STUDIES on the TOXICITY and TERATOGENICITY of SACCHARIN

SUMMARY and CONCLUSIONS

Saccharin, administered either in acetone or DMSO, produced significant increases ($P < 0.05$) in embryo mortality. The highest dose tolerated without a significant increase in embryo mortality was 30 mg/kg administered in the air cell prior to incubation. Employing yolk administration with acetone as the solvent, a dose level up to 75 mg/kg did not significantly increase embryo mortality; however, when all doses were combined and tested by chi-square analyses, significant increases were found for both yolk administration times. A level of 160 mg/kg was tolerated when administered in dimethyl sulfoxide. Levels above this (200 mg/kg) produced significant increases in embryo mortality when injected in the air cell at 0 hours. A much lower dose of 20 mg/kg produced a significant increase in embryo mortality when 96 hour embryos were treated. A dose of 10 mg/kg was tolerated without producing a significant mortality increase in 96 hour embryos. Yolk administration at 96 hours employing dimethyl sulfoxide as a solvent for saccharin did not produce a significant increase in embryo death.

The only significant ($P < 0.05$) increase in the incidence of abnormalities was noted with the 75 mg/kg dose level of saccharin administered in dimethyl sulfoxide in the egg cell prior to incubation. The abnormalities noted in association with this dose level were found on histopathological examination of randomly selected embryos. These findings include renal tubular degeneration and the presence of granulation tissue in the renal tubule and increased vacuolization of the liver. Linear regression analyses employing log dose vs the probit of abnormality incidence produced a significant linear relationship for the air cell administration of dimethyl sulfoxide solubilized saccharin at 96 hours incubation. The yolk administration of this material at 96 hours showed a linear relationship which was significant at the 0.1 level of probability. These data suggest that saccharin, administered under the conditions of these studies was teratogenic.

Chicks maintained to six months of age after having been injected with saccharin in dimethyl sulfoxide exhibited a reduction in body weight and feed consumption, and a delay in sexual maturity for females. The dose level associated with these effects was 200 mg/kg.

GENERAL PROCEDURES

The protocols as specified under FDA Contract #71-330 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (pre-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range- finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

The remaining birds in each group were maintained to 6 months of age and then sacrificed.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
2. Linear regression analyses + chi square test of linearity.
   a. % response against dose
   b. % response against log dose
   c. log % response against dose
   d. arcsin transformation against dose
   e. arcsin transformation against log dose
3. Log dose against Probit using Finney's maximum likelihood method.
   a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
4. One-way analyses of variance.
5. Linear regression with replication.

Acid saccharin (71-04) was solubilized in two different solvents for use in the test protocols. Acetone was employed with tests of air cell - 0 hr injections and for both yolk (0 & 96 hrs) administration to provide a maximum dose of 75 mg/kg (3.75 mg/egg). Dimethyl sulfoxide (DMSO) was used for air cell administration at 0 and 96 hours, and for yolk injections at 96 hours to provide a maximum dose level of 200 mg/kg (10 mg/egg).

MORTALITY

The mortality data for the acetone and DMSO solutions of saccharin are shown in Tables 1 - 6. Acetone solubilized saccharin produced significant increases in embryo mortality in comparison with the solvent control groups for each of the three test protocols in which this solution was employed (Tables 1 - 3). Chi-square analyses of mortality data showed statistical significance ($P < 0.05$) for dose levels of 45 mg/kg and above for the air cell-0 hour groups (Table 7).

When administered in the yolk at either 0 or 96 hours, the acetone solution of saccharin produced significant chi-square values when all doses were considered (Table 7). The lower dose levels of 10 & 20 mg/kg when injected into the yolk prior to incubation produced significantly ($P<0.05$) less mortality than the acetone alone (Table 7).

Injection of DMSO solutions of saccharin also resulted in increased embryo mortality in two of the three protocols where these solutions were used (Tables 4 - 6).

Chi-square analyses of these data indicated statistically significant values ($P < 0.05$) for the two air cell administration times (Table 8). A dose level of 200 mg/kg significantly increased embryo mortality in those embryos receiving the DMSO solution in the air cell prior to incubation. Dose levels of 1 and 2 mg/kg produced significantly less mortality than the solvent controls employing air cell administration after 96 hours incubation, while dose levels of 20 and 40 mg/kg significantly increased embryo mortality (Table 8). Yolk administration of DMSO solutions of saccharin in 96 hour embryos did not significantly increase embryo mortality.

Linear regression analyses of log dose vs probit of mortality produced a significant linear relationship for only the air cell-0 hour series when acetone was employed as the solvent. LD-50 estimates from these data indicated a dose level of 304.4 mg/kg for the acetone solution injected in the air cell prior to incubation (Table 9). Similar analyses of saccharin administration in DMSO again indicated a significant relationship between log dose and probit of mortality for only the air cell-0 hour series. LD-50 estimates from these data indicated a value of 196.3 mg/kg (Table 10).

TERATOLOGY

The incidence of abnormal embryos among the test protocols and two solvents are shown in Tables 1 - 6. Probit analyses of these data did not demonstrate a significant linear relationship for any of the three protocols where acetone was employed as the solvent (Table 13). DMSO solutions of saccharin produced a significant linear relationship ($P < 0.05$) for the air cell-96 hour series (Table 14). The LD-50 estimate from these data was relatively high with a value of 48964.8 mg/kg. Yolk administration after 96 hours incubation exhibited a linear relationship between log dose and probit of abnormality incidences which was significant at the 0.1 level of probability and an LD-50 estimate of 5350.3 mg/kg (Table 14).

Chi-square tests of the abnormality data showed a significant increase in abnormal embryos for the 75 mg/kg saccharin in DMSO when this level was administered prior to incubation in the air cell (Tables 11 & 12). There were no significant chi-square values for the data on H-L-S-V abnormalities (Tables 15 & 16).

The specific teratology findings are shown in Table 17. Air cell-0 h ur administration of saccharin in DMSO at a dose level of 75 mg/kg produced a significant chi-square value due to the histopathological results (Table 17). These findings showed involvement of renal tubules (degeneration) and increased vacuolization in liver cells.

POST HATCH DATA

In chicks maintained for 6 months post-hatch, a dose level of 200 mg/kg produced a depression in weight of male chicks at six weeks of age and in both males and females at six months (Table 18). These findings also suggest a delay in sexual maturity in females at this dose level (Table 18).

TABLE I

SACCHARIN
in ACETONE
AIR CELL - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
75.0	110	23.63	26	3.63	4	5.45	6	0.90	1			1.81	2	2.72	3						
60.0	110	27.27	30	0.90	1	0.90	1	0.90	1												
45.0	110	22.72	25	2.72	3	2.72	3	2.72	3							0.90	1				
30.0	110	12.72	14	3.63	4	1.81	2	1.81	2												
20.0	100	14.00	14	2.00	2	2.00	2	1.00	1			1.00	1								
0.0	110	6.36	7	0.90	1	0.90	1							0.90	1						
drilled	194	9.27	18	1.03	2	0.00	0									1.03	2				
untreated	492	10.16	50	1.42	7	1.62	8	1.01	5	0.20	1	0.40	2					0.20	1		

SUMMARY - ALL DOSE LEVELS

540		20.19	109	2.59	14	2.59	14	1.48	8			0.56	3	0.56	3	0.19	1				

TABLE 2

SACCHARIN
IN ACETONE
YOLK - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
75.0	116	42.24	49	1.72	2	1.72	2	0.86	1					0.86	1						
60.0	117	41.02	48	1.70	2	1.70	2	1.70	2												
45.0	117	36.75	43	0.00	0	0.00	0														
30.0	59	27.11	16	0.00	0	0.00	0														
20.0	107	15.88	17	1.86	2	1.86	2	0.93	1			0.93	1			0.93	1				
10.0	56	14.28	8	0.00	0	0.00	0														
0.0	116	31.03	36	0.00	0	0.00	0														
pierced	209	60.76	127	1.43	3	0.95	2	0.95	2							0.47	1				
untreated	492	10.16	50	1.42	7	1.62	8	1.01	5	0.20	1	0.40	2					0.20	1		

SUMMARY - ALL DOSE LEVELS

572	31.64	181	1.05	6	1.05	6	0.70	4		0.17	1	0.17	1	0.17	1		

TABLE 3

SACCHARIN
IN ACETONE
YOLK - 96 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
75.0	30	20.00	6	3.33	1	3.33	1	3.33	1												
60.0	30	26.66	8	3.33	1	0.00	0									3.33	1				
45.0	30	20.00	6	0.00	0	0.00	0														
30.0	30	6.66	2	0.00	0	0.00	0														
10.0	30	0.00	0	3.33	1	3.33	1	3.33	1												
0.0	30	13.33	4	0.00	0	0.00	0														
—pierced	130	43.84	57	2.30	3	0.76	1	0.76	1									1.53	2		
untreated	492	10.16	50	1.42	7	1.62	8	1.01	5	0.20	1	0.40	2					0.20	1		

SUMMARY - ALL DOSE LEVELS

150	14.67	22	2.00	3	1.33	2	1.33	2						0.67	1				

TABLE 4

SACCHARIN
in DIMETHYL SULFOXIDE
AIR CELL - 0 HRS

Dose, ppm	No. Fertile	Mortality		Abnormal				Abnormalities by category											
				Total		H-S-V-L		Head		Skeletal		Viscera		Limbs		Struc-tural		Toxic Response		Functional	
		%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#
200.0	49	77.55	38	6.12	3	2.04	1	2.04	1							6.12	3				
160.0	50	48.00	24	8.00	4	4.00	2	4.00	2									4.00	2		
80.0	99	45.45	45	1.01	1	1.01	1					1.01	1								
75.0	33	21.21	7	36.36	12	0.00	0														
40.0	99	28.28	28	4.04	4	3.03	3	3.03	3							1.01	1				
20.0	100	34.00	34	2.00	2	1.00	1	1.00	1									1.00	1		
10.0	99	28.28	28	0.00	0	0.00	0														
0.0	136	36.02	49	2.20	3	0.73	1	0.73	1							0.73	1			0.73	1
drilled	194	9.27	18	1.03	2	0.00	0							1.03	2						
untreated	492	10.16	50	1.42	7	1.62	8	1.01	5	0.20	1	0.40	2					0.20	1		

SUMMARY - ALL DOSE LEVELS

529	38.56	204	4.91	26	1.51	8	1.32	7			0.19	1			0.76	4	0.57	3		

TABLE 5

SACCHARIN
in DIMETHYL SULFOXIDE
AIR CELL - 96 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
40.0	97	56.70	55	6.18	6	0.00	0											1.03	1	1.03	1
20.0	183	49.72	91	3.27	6	3.82	7	1.63	3			1.63	3	0.54	1	0.54	1				
10.0	100	25.00	25	4.00	4	4.00	4	2.00	2			2.00	2			1.00	1				
5.0	99	28.28	28	2.02	2	2.02	2	1.01	1			1.01	1								
2.0	100	11.00	11	0.00	0	0.00	0														
1.0	97	15.46	15	1.03	1	1.03	1	1.03	1												
0.0	155	37.41	58	1.93	3	2.58	4	1.29	2			1.29	2								
drilled	134	7.46	10	0.00	0	0.00	0														
untreated	492	10.16	50	1.42	7	1.62	8	1.01	5	0.20	1	0.40	2					0.20	1		

SUMMARY - ALL DOSE LEVELS

676	33.28	225	2.81	19	2.07	14	1.04	7		0.89	6	0.15	1	0.30	2	0.15	1	0.15	1

TABLE 6

SACCHARIN
in DIMETHYL SULFOXIDE
YOLK - 96 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Struc-tural %	Struc-tural #	Toxic Response %	Toxic Response #	Functional %	Functional #
80.0	154	46.10	71	3.24	5	0.00	0											2.59	4	0.64	1
60.0	160	40.62	65	1.87	3	0.62	1					0.62	1					1.25	2		
40.0	151	45.69	69	0.66	1	0.66	1					0.66	1								
20.0	160	45.62	73	0.00	0	0.00	0														
10.0	157	52.86	83	0.63	1	0.00	0									0.63	1				
0.0	118	43.22	51	0.00	0	0.00	0														
pierced	130	43.84	57	2.30	3	0.76	1	0.76	1									1.53	2		
untreated	492	10.16	50	1.42	7	1.62	8	1.01	5	0.20	1	0.40	2					0.20	1		

SUMMARY - ALL DOSE LEVELS

782	46.16	361	1.28	10	0.26	2			0.26	2		0.13	1	0.77	6	0.13	1

TABLE 7

SACCHARIN in ACETONE
CHI-SQUARE ANALYSES of MORTALITY

Dose Level mg/kg	Air Cell	Yolk	
	0 hrs	0 hrs	96 hrs
10.0	-	4.72*(less)	2.41
20.0	2.60	6.24*(less)	-
30.0	1.90	0.13	0.19
45.0	10.57*	0.61	0.12
60.0	15.73*	2.11	0.94
75.0	11.55*	2.67	0.12
All Doses (DF)	23.84*(5)	32.91*(6)	11.69*(5)

* Probability $<$ 0.05 - 0.005.

TABLE 8

SACCHARIN in DIMETHYL SULFOXIDE
CHI-SQUARE ANALYSES of MORTALITY

Dose Level mg/kg	Air Cell		Yolk
	0 hrs	96 hrs	96 hrs
1.0	-	12.93*(less)	-
2.0	-	20.18*(less)	-
5.0	-	1.86	-
10.0	1.23	3.72	2.14
20.0	0.03	4.67*	0.08
40.0	1.23	8.21*	0.08
60.0	-	-	0.10
75.0	2.01	-	-
80.0	1.75	-	0.12
160.0	1.72	-	-
200.0	23.30*	-	-
All Doses (DF)	49.75* (7)	86.66* (6)	5.21 (5)

* Probability $\leq$ 0.05 - 0.005

TABLE 9

SACCHARIN in ACETONE
PROBIT ANALYSES - MORTALITY

	Air Cell	Yolk	
	0 hrs mg/kg	0 hrs mg/kg	96 hrs mg/kg
LD-30 (Range)	116.7 (182004.0 - 70.8)	NS	NS
LD-50 (Range)	304.4 (* - 123.1)	NS	NS
LD-70 (Range)	793.9 (* - 207.2)	NS	NS
LD-90 (Range)	3169.9 (* - 4.34.6)	NS	NS

* = number too large for computer field.

TABLE 10

SACCHARIN in DIMETHYL SULFOXIDE
PROBIT ANALYSES - MORTALITY

Dose Level mg/kg	Air Cell		Yolk
	0 hrs	96 hrs	96 hrs
LD-30 (Range)	128.2 (196.3 - 53.7)	NS	NS
LD-50 (Range)	196.3 (193.3 - 199.3)	NS	NS
LD-70 (Range)	300.5 (293.1 - 308.0)	NS	NS
LD-90 (Range)	555.6 (533.1 - 579.2)	NS	NS

TABLE II

SACCHARIN in ACETONE
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level mg/kg	Air Cell	Yolk	
	0 hrs	0 hrs	96 hrs
10.0	-	0.00	0.00
20.0	0.01	0.60	-
30.0	0.82	0.00	0.00
45.0	0.26	0.00	0.00
60.0	0.51	0.50	0.00
75.0	0.82	0.50	0.00
All Doses (DF)	3.76 (5)	6.22 (6)	3.05 (5)

TABLE 12

SACCHARIN in DIMETHYL SULFOXIDE
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk
	0 hrs	96 hrs	96 hrs
1.0	-	0.00	-
2.0	-	0.65	-
5.0	-	0.17	-
10.0	0.81	0.35	0.02
20.0	0.12	0.18	0.00
40.0	0.18	2.02	0.02
60.0	-	-	0.83
75.0	34.20*	-	-
80.0	0.04	-	2.31
160.0	1.98	-	-
200.0	0.73	-	-
All Doses (DF)	93.04* (7)	9.86 (6)	10.96 (5)

* Probability $<$ 0.05 - 0.005.

TABLE 13

SACCHARIN IN ACETONE
PROBIT ANALYSES - ABNORMALITIES

	Air Cell	Yolk	
	0 hrs	0 hrs	96 hrs
LD-30	NS	NS	NS
LD-50	NS	NS	NS
LD-70	NS	NS	NS

TABLE 14

SACCHARIN in DIMETHYL SULFOXIDE
PROBIT ANALYSES - ABNORMALITIES

	Air Cell		Yolk
	0 hrs	96 hrs	96 hrs
			Sig. $P < 0.1$
LD-30 (Range)	NS	5487.9 (* - 336.0)	1717.6
LD-50 (Range)	NS	48964.8 (* -1050.9)	5350.3
LD-70 (Range)	NS	436877.3 (* -1050.0)	16666.2
LD-90 (Range)	NS	10298636.0 (*-16714.7)	85973.0

* = number too large for computer field.

TABLE 15

SACCHARIN in ACETONE
CHI-SQUARE ANALYSES of H-L-S-V ABNORMALITIES

Dose Level mg/kg	Air Cell	Yolk	
	0 hrs	0 hrs	96 hrs
10.0	-	0.00	0.00
20.0	0.01	0.00	-
30.0	0.00	0.00	0.00
45.0	0.26	0.00	0.00
60.0	0.51	0.50	0.00
75.0	0.82	0.50	0.00
All Doses (DF)	3.15 (5)	5.78 (6)	4.05 (5)

TABLE 16

SACCHARIN in DIMETHYL SULFOXIDE
CHI-SQUARE ANALYSES of H-L-S-V ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk
	0 hrs	96 hrs	96 hrs
1.0	-	0.00	-
2.0	-	0.65	-
5.0	-	0.17	-
10.0	0.03	0.35	0.00
20.0	0.25	0.02	0.00
40.0	0.69	0.61	0.02
60.0	-	-	0.02
75.0	0.59	-	-
80.0	0.24	-	0.00
160.0	0.83	-	-
200.0	0.00	-	-
All Doses (DF)	7.26 (7)	7.60 (6)	3.80 (5)

Sheet 1

TABLE 17

SACCHARIN
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Untreated Control	492	7	1	anophthalmia - bilateral
			1	malposition
			1	anophthalmia-bilateral; encephalocele; agenesis-maxilla; celosomia-abdomen
			1	exencephaly
			1	anophthalmia-bilateral; agenesis-maxilla
			1	anophthalmia-bilateral; microcephaly-head; acrania; abnormal shortening-maxilla; abnormal flexion-mandible; scelerosis-spinal nerve; agenesis-neck
			1	fusion failure-abdomen
Drilled Control - 0 hrs	194	2	2	dwarfism
Drilled Control - 96 hrs	134	0	0	
Pierced Control - 0 hrs	209	3	1	anophthalmia-bilateral; dysgnathia-beak
			1	microphthalmia-unilateral; abnormal shortening-maxilla
			1	hypopigmentation - down
Pierced Control - 96 hrs	130	3	1	malposition
			1	hemorrhage
			1	abnormal shortening-maxilla

TABLE 17

SACCHARIN
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Saccharin in Acetone				
Air Cell - 0 hrs 75.0 mg/kg	110	4	1	contracture-toe; fusion failure - abdomen
			1	anophthalmia-bilateral; dysallilognathia-beak; aplasia-wing
			1	celosomia-abdomen
			1	malrotation-hindlimb
60.0	110	1	1	microphthalmia-bilateral; agenesis-maxilla
45.0	110	3	1	acrania
			1	anophthalmia-bilateral; acrania; agenesis-maxilla; dwarfism
			1	edema-neck
30.0	110	4	1	exencephaly
			1	enophthalmia-bilateral; agenesis-maxilla
			1	granulation - liver
			1	malacia-spinal cord; granulation-renal tube
20.0	100	2	1	anophthalmia-unilateral; dysgnathia-beak
			1	celosomia - abdomen
0.0	110	1	1	edema-hindlimb, unilateral

TABLE 17

Sheet 3

SACCHARIN
TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Saccharin in Acetone					
Yolk - 0 hrs	75.0 mg/kg	116	2	1	cyclopia-head; dysallilognathia-beak
				1	malrotation - hindlimb, unilateral
	60.0	117	2	1	edema - neck
				1	agenesis - head
	45.0	117	0	0	
	30.0	59	0	0	
	20.0	107	2	1	dwarfism
				1	edema - neck, celosomia - abdomen
	10.0	56	0	0	
	0.0	116	0	0	
Saccharin in Acetone					
Yolk - 96 hrs	75.0	30	1	1	acrania
	60.0	30	1	1	dwarfism
	45.0	30	0	0	
	30.0	30	0	0	
	10.0	30	1	1	acrania
	0.0	30	0	0	

Sheet 4

TABLE 17

SACCHARIN
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Saccharin in Dimethyl Sulfoxide				
Air Cell - 0 hrs 200.0 mg/kg	49	3	2	dwarfism
			1	microphthalmia-bilateral, dwarfism
160.0	50	4	2	hypopigmentation - down
			2	edema - neck
80.0	99	1	1	fatty metamorphosis - liver
75.0	33	12	4	degeneration - renal tubule
			2	extracellular space alteration - lung
			1	granulation tissue - renal tubule
			4	vacuolization - liver
			1	vacuolization - liver; granulation tissue - renal tubule
40.0	99	4	1	agenesis - head
			1	dysgnathia - beak
			1	dwarfism
			1	edema - neck
20.0	100	2	1	hemorrhage - head
			1	edema - neck
10.0	99	0	0	
0.0	136	3	1	ataxia
			1	dwarfism
			1	agenesis - head

TABLE 17

Sheet 5

SACCHARIN
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Saccharin in Dimethyl Sulfoxide				
Air Cell - 96 hrs 40.0 mg/kg	97	6	4	granulation tissue - renal tubule
			1	ataxia
			1	hypopigmentation - down
20.0	183	6	1	ectromelia - hindlimb, unilateral; celosomia - abdomen
			1	celosomia - abdomen
			1	dwarfism
			1	anophthalmia-bilateral; acrania; dysgnathia - beak
			1	microphthalmia - unilateral; encephalocele
			1	anophthalmia-bilateral; acrania; agenesis-maxilla; celosomia - abdomen
10.0	100	4	1	anophthalmia-bilateral; dwarfism
			2	celosomia - abdomen
			1	anophthalmia-bilateral; dysgnathia - beak
5.0	99	2	1	celosomia - abdomen
			1	anophthalmia - unilateral
2.0	100	0	0	
1.0	97	1	1	edema - neck
0.0	155	3	1	celosomia - abdomen
			1	anophthalmia-unilateral; dysgnathia beak
			1	anophthalmia-bilateral; edema-neck; abnormal shortening-maxilla; celosomia-abdomen

TABLE 17

SACCHARIN

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	SPECIFIC FINDINGS DESCRIPTION
Saccharin in Demethyl Sulfoxide				
Yolk - 96 hrs 80.0 mg/kg	154	5	1	ataxia
			3	hypopigmentation - down
			1	malposition
60.0	160	3	2	hypopigmentation - down
			1	celosomia - abdomen
40.0	151	1	1	celosomia - abdomen
20.0	160	0	0	
10.0	157	1	1	dwarfism
0.0	118	0	0	

TABLE 18

POST-HATCH DATA
SACCHARIN in DMSO

INJECTION DATE: 9/27/71

LABEL	DOSE mg/kg	AVE. AGE at SEXUAL MATURITY	BODY WEIGHT					AVERAGE FEED CONSUMPTION	
			INITIAL gm	6 wks gm		6 mos gm		6 wks gm	6 mos kg
				M	F	M	F		
201	10.0	144	42.3	469	408	1816	1589	943	13.28
202	20.0	144	40.3	496	450	1893	1816	1018	12.77
203	40.0	146	41.4	447	396	1893	1816	970	12.15
204	80.0	141	41.7	469	382	1857	1589	898	11.85
205	200.0	153	40.4	336	415	1557	1285	798	13.39
206	DMSO (0.02 ml)	140	41.7	453	396	1816	1589	927	13.24
207	" (0.05 ml)	145	41.3	500	401	1666	1421	947	10.93
209	untreated	145	40.0	473	464	1875	1780	1057	12.80